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CONFERENCE ON THE PROBLEM OF
PHYSIOLOGICAL REGENERATION

By I. A. Utkin and N. P. Bochkov

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CONFERENCE ON THE PROBLEM OF PHYSIOLOGICAL REGENERATION
by I.A. Utkin and N.P. Bochkov

[Following is the translation of an article
by I.A. Utkin and N.P. Bochkov entitled
"Konferentsiya" (English version above) in
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By the decision of the Presidium of Academy of
Medical Sciences, USSR, a conference was held concerning
the problems of physiological regeneration from the 11th
to the 14th of May, 1959 in Sukhumi in the Institute of
Experimental Pathology and Therapy, AMS, USSR. Over 100
persons from Moscow, Leningrad, Sbilisi, Sukhumi, Frunze
and other cities of the USSR took part in the activities
of the conference. More than 40 articles were heard and
discussed in the sessions.

The conference was opened by the members of the
Presidium of the AMS, USSR, Prof. V.Kh. Vasilenko, who

emphasized the importance of theoretical studies for modern practical medicine and the special actuality of physiological regeneration problem.

The First Session concerned theoretical problems and methodologies in the study of physiological regeneration.

L.D. Lioznev (Moscow) in the paper "Principle problems in studying physiological regeneration" noted the physiological regeneration is a regular process, inseparable from body activity. The physiological regeneration processes can take place at different levels: 1) organic; 2) tissue; 3) intracellular. The author emphasized the relation of the development of physiological and reparative regeneration problems. Physiological regeneration is insufficiently studied in relation to some pathological conditions, and in its evolutionary aspect. The author considered that the problem of cell multiplication, although it stands close to the problem of physiological regeneration, represented an independent problem.

S.I. Shchelkunov (Leningrad) in the paper "On the

regularities of histogenesis and tissue regeneration" noted the similarities and differences of these processes and emphasized the role and meaning of mitosis and amitosis in them. According to his opinion, the hereditary information, providing the histologic tissue determinancy, is accomplished by mitotic division. During amitosis the transformation of hereditary information takes place. The alternation of different division methods of tissue cells, seems to be much more complicated than it was thought up to the present.

Experimental study results on the regularities of cell multiplication cultivated outside the organism were reported by S.Ya. Zalkind (the speaker), V.G. Zaslavskiy, T.G. Orlova and N.V. Borisoglebskaya (Moscow). The mitotic activity of malignant HeLa strain and HEP2 cultures is much higher than that of strain SOTS and the kidney tissue from rhesus monkeys. Each strain has its own characteristics of mitotic activity dynamics. The height of mitotic activity depends on the concentration of primary suspension and medium composition. Daily mitotic rhythm was not noted in any of the cultures.

There were many abnormal mitotic forms in the tissue cultures. The assumption was expressed and proven that the cells in the culture can also multiply in amitotically.

In the review paper, "On the application of tritium in thymidine composition in cytoradiographic studies of cell division and physiological regeneration" G.S. Strelin (Leningrad) noted the great prospect of using preparations containing in their composition hydrogen isotope - tritium. Great methodological possibilities and important advantages of tritium in cytoautoradiographic studies are related with the fact that hydrogen enters into the composition of overwhelming majority of organic compounds and consequently any substance can be labelled, which takes part in tissue and cell metabolisms. Further, the track length of particles formed when tritium disintegrates is considerably smaller than that in the usually applied isotopes in autoradiography. As a consequence of this, the location of the tracks very accurately corresponds to the labelled compound location in the cell or the tissue, i.e., the accuracy of cytoautoradiographic studies considerably increases.

An interesting paper "On chemical differentiation of some "cambiogenetic" tissues in physiological regeneration process" was presented by L.N. Zhinkin (Leningrad). Autoradiography permitted demonstration of the nature of protein metabolism in cornified epithelium, connective tissue and erythrocytes in the process of their differentiation. Author dwelled on the complex interrelations between cell proliferation, metabolism and specific differentiation. The appearance of specific differentiation is related to the loss in cells of mitotic division. Nucleic metabolism is weakened with the appearance of specific differentiation of cells.

A.A. Braun (Frunze) in the paper "On the influence of the factors of external and internal medium of the organism on physiological epidermis regeneration" discussed the problem of interrelations of the break-down and restoring phases in epidermis self-restoration processes. His suggested method for registering the number of cell rows of growth and granular layers and their thickness together with a consideration of mitotic activity permits judgement on the physiological regeneration processes.

The restoration phase of physiological regeneration is augmented with local action of ceresin, paraffin, ultra-violet radiation, and is weakened under the influence of X-irradiation and denervation of symathetic nerves.

The problem of the ways epithelial cells die in the in the small intestine was discussed by N.P. Bochkov (Sukhumi) in the paper "On the disputable problems of physiological epithelial regeneration is small intestines". On the basis of the analysis of the literature and his own different experiments the author came to the conclusion that in the papers by M.I. Razimov and Z.M. Gadzhiyev are described not "the elements of functional morphology of small intestine", but the artifacts appearing with fixing, and mounting the material. On estimating the destructive phase of physiological regeneration of the small intestine epithelium, it is important to consider the possibility of the origination of artifacts. Artifacts do not appear, if the material is fixed in a cold fixative or stretched.

Those who appeared in the debates (A.N. Studitskiy, S.Ya. Zalkind, L.N. Zhinkin, A.A. Braun, L.D. Liozner,

V.P. Mikhaylov, A.S. Lezhava, G.S. Strelin and I.A. Utkin) noted the great value of the reported data. The radio-autographic study method of physiological regeneration processes deserved special approval.

In the Second and Third Sessions several reports were heard, on the problem of mitotic activity regulation in the body and the methods of quantitative estimation of proliferative and regenerative processes.

V.N. Dobrokhotoy (Moscow) in a review "On the present position of the problem of daily periodic mitotic cell division in animals" gave the status of this problem and planned the problems and methods for its further development, as one of the ways of studying the mechanisms of physiological regeneration regulation, the author critically examined the methodological procedures for the development of the problem of daily periodic mitotic cell division. The following problems were subject to detailed analysis: a) daily rhythm of cell multiplication in tissues with a high mitotic coefficient; b) the synchronous feature of daily changes of mitotic coefficients in different tissues; c) the value of periodically

changing factors of external medium in the determination of daily changes of the mitotic activity; d) the characteristics of internal factors causing periodic changes in mitotic activity.

In the paper by L.D. Lioznev and V.F. Sidorov (Moscow) "On the physiological regeneration of liver" experimental data was given for the study of mitotic activity in mouse liver through one day at three hour intervals. It was found that in mouse liver there is a daily periodicity with a mitosis maximum (6 per 1,000 cells on the average) from three to nine hours. According to the authors' opinion, the self-restoration of liver cells at the expense of mitotic divisions can occur in 100 days.

V.V. Kozlov (Leningrad) in the paper "On the problem of the daily rhythm mechanism of mitotic activity" reported the results of his study of the role of adrenal glands in daily rhythmic cell multiplication. Although with removal of adrenals in rats average daily mitotic activity did not change, the amplitude of its oscillations at different time of the day was considerably decreased in

the corneal epithelium in animals without suprarenals.

The results of experimental work "Daily and seasonal periodicity of mitosis in corneal epithelium of laboratory animals" were reported by L.P. Kosichenko (Sukhumi). She discovered specific differences in the seasonal and daily dynamics of mitosis activity in mice, rats and guinea pigs. Daily and seasonal mitosis periodicity was most sharply expressed in mice. In rats the daily fluctuations of mitotic activity are also clearly expressed, although less sharply. However, no noticeable changes were recorded in the mean day mitotic number in different seasons of the year. In guinea pigs daily and seasonal periods were weakly expressed. The character of daily mitotic periodicity in mice and rats is the same for all seasons of the year.

O.I. Epifanova (Moscow) presented experimental material on the effect of folliculin and progesterone on mitotic activity of the mucous membrane epithelium in the mouse uterus. Estrogen in castrated mice stimulates mitotic activity of uterine epithelium for several days. Further saturation of organism with a hormone leads to the

decrease of the dividing cell number. The discontinuation of preparation administration causes an increase of mitotic activity. Progesterone likewise increases mitotic activity, but if folliculinization is made before hand, it almost has no effect on it. The functional condition of the organ is of great importance in epithelial reaction to hormone introduction.

A similar arrangement of experiments was made by S.S. Laguchev (Moscow). In his paper "The effect of ovarian hormones on the mitotic activity of mammary gland epithelium" he noted that mitotic division in mammary gland epithelium is absent in castrated female mice. The introduction of folliculin stimulates cell division, although it does not restore the normal condition of glands, because of the slowing down of organo-specific differentiation. Discontinuation of preparation administration leads to a decrease in the mitotic coefficient. Progesterone stimulates the mitotic activity of mammary gland epithelium, but not as strongly as folliculin.

The paper of I.A. Utkin, M.V. Avkzhian, Yu.P. Butnev and G.G. Kuznetsova (Sukhumi) "In vitro study of

cell division corneal epithelium, incubated outside the organism" concerned experimental search for additional ways of studying the cell division mechanism and its regulating factors. The authors subjected the mitotic activity of the corneal epithelium to a comparative study in body conditions and after incubation of eyes in vitro in media of different composition. In enucleated eyes were incubated in a Warburg apparatus with 37° C from 15 minutes to 4 hours. It was established that during the incubation the beginning of cell division is sharply retarded, although the course of mitosis itself and its rate are essentially not disturbed. The mitotic rate was not noticeably changed with considerable fluctuations in pH agent (from 6.7 to 7.9), and also with the addition to it of insulin, adrenalin, glucose and glycogen. Distinct retarding effect on mitosis was produced by poisons which disturbed mitotic energy (cyanides, sodium fluoride, moniodoacetic acid, 2,4 dinitrophenol).

N.P. Bochkov (Sukhumi) reported on the mitotic duration and the rate of physical regeneration in rat intestinal epithelium. Applying the method of total-body

gamma-irradiation, the author found that the rate of mitosis in intestinal epithelium is 33 minutes and not 68, as it was shown by Leblon and Stevens with the aid of colchicine. Complete rejuvenation of epithelial layer through mitosis can occur in 24.4 hours in rats.

O.T. Movchan (Sukhumi) made the report "Mitotic activity of corneal epithelium in white rats and white mice with complete starvation". The author showed that mitotic activity with starvation at first increases, and later decreases. Interrelation was discovered between the weight of the animals and the variation characteristics of mitotic activity with starvation: the lower was the weight of the animals, the slower was the rise of mitotic activity at the beginning of starvation and there was a sharper decrease of mitotic activity at the end of starvation. Keeping of rats at a lower temperature speeds up the retardation of mitotic activity. The mitotic rate and daily periods are not disturbed because of starvation.

"On the study of mitotic activity of the mucous membrane epithelium in the oral cavity and the intestine in some mammals at different times after feeding" was

reported by G.S. Uspenskaya and A.B. Kalmeniek (Moscow). It was established, that feeding of variously starving rabbits and guinea pigs changes the mitotic activity of mucous membrane epithelium of the cheek-pouch and the intestine, whereupon the maximum rise of mitotic activity of mucous membrane epithelium in intestine coincides in time ($1/2$ to one hour after feeding) with its maximum decrease in the mucous membrane epithelium of the cheek. The authors viewed the data of their study as preliminary.

Ye.V. Zybyna (Leningrad) in the article "Mitosis and amitosis in giant trophoblast cells and in decidua cells" presented data on the nature of trophoblast and decidua cells in rats. The decidua cells become polyploid rather fast, it seems through endomitosis and nuclear confluence. Nuclear fragmentation begins with the attenuation of mitotic activity in them. They degenerate by the 15th day of embryo development. Phagocytosis, mitotic multiplication and polyploidy at the expense of endomitosis are characteristic for trophoblast cells. After the 13th day of pregnancy the fragmentation of giant cells was recorded.

L.N. Moralev (Kursk) devoted his report to destructive and regenerate effects in the pancreas local disturbances of blood circulation. The author with an original method (placing movable wire ligatures on the vessels) caused blood circulation disturbances of different degrees and duration. Then he succeeded in restoring the blood flow. With slight ischemia (up to 6 hours) the destructive processes touched several cells in exocrinal and endocrinal parts of the gland and reminded one of the processes of G.V. Yasvoin "dark cell" formation. Karyokinetic figures were observed in exocrinal part. The discovered effects were compared by the author with physiological organ regeneration. With long disturbance of blood circulation a picture is exposed analogous to that which takes place in different forms of trauma.

In the discussion of reports in two sessions, 20 persons took part. Some recommendations were made on further development of methods for quantitative estimation of cell multiplication and physiological regeneration. In addition to this it is necessary to consider the

different forms of tissue-self rejuvenation. The importance of studying the destructive phase of physiological regeneration, especially in the intestine was emphasized.

The Fourth Session in the first half of the fourth session the final reports on mitotic activity were heard.

In the article of S.Ya. Zalkind and N.V. Borisoglebskaya (Moscow) "The mitotic activity of organism in the development process of immunity to poliomyelitis" the results were reported of the study of mitotic activity in the spleen and the corneal epithelium in rats with their immunization by poliomyelitis virus or vaccine. In the process of immunogenesis the mitotic activity increases in the spleen and also in the cornea. The possibility was noted of practical application of the mitotic activity determination method for the determination of organism reaction and the vaccine properties.

Ya. Ye. Khesin, O.F. Sarycheva, F.V. Voroniva, E.R. Pille, Yu.N. Mastjukova and K.S. Blinnikov (Moscow) presented material on the karyometric study of monolayer tissue cultures in normal conditions and under the influence of some viruses. With the influence of different

viruses already after 4-6 hours an increase of the average nuclear size and modulus displacements of the variational curve had been observed. The increase of nuclear size is not the cause of direct virus multiplication in the cell, but reflects the change of physiochemical properties of the nuclear colloids, which leads to swelling. Together with an increase of the average nuclear size, the number of multi-nuclear symplastic formations and abnormal mitoses increases.

Experimental results of the relative effect of neurotropic substances on mitotic activity in corneal epithelium were reported by Ye.S. Tsareva (Stalino). Neurotropic substances were administered to mice 1-3 hours before sacrifice, and the mitotic activity in corneal epithelium was examined. Rise of sympathetic nervous system tonus with the introduction of adrenalin causes a decrease of mitotic activity, however the decrease after the introduction of ergotin increases the cell division. In relation to parasympathetic nervous system an inverse relationship has been obtained: introduction of procerin increase the mitotic activity, atropine - decreases.

In the discussion of these reports the problem on cell division regulation in the organism was broached (I.A. Utkin, A.N. Studitskiy, S.Ya. Zalkind, G.S. Strelin, S.I. Shchelkunov). Those who spoke assume that it is accomplished through tissue metabolism. The reports of Ye.S. Tsareva and L.I. Omel'yanenko received favorable evaluation on the whole, but there were made some critical remarks in relation with the methodology used by these authors.

In the second half of the report session articles were heard dealing with the relation of repair and physiological regeneration.

N.I. Grigor'ev (Leningrad) made a report on the relationship between physiological and repair regeneration of intestinal type epithelium. By examples of mammals, birds, tailed and non-tailed amphibia and bony fish a direct relation was shown between the intensity of physiological and repair regeneration in intestine epithelium, together with that, in liver and gall bladder the physiological regeneration was weakly expressed, but the reparative regeneration was clearly exposed. The author re-

lated this with the fact that the liver and gall bladder epithelium ontogenetically forms from the front entodermic part of the intestine section. Similarity was noted between physiological and reparative regeneration of one and the same tissue. With reparative and also with physiological regeneration the meaning of "cambio-genetic" sections was preserved.

B.P. Solopayev (Sukhumi) reported on the experimental studies on the problem of repair regeneration of normal and pathologically changed liver in mammals. It was established that monkey liver regenerates well. In rabbits even the chirotoically changed liver regenerates. Reparative regeneration of white rat liver is reinforced under the influence of different bile flow stimulating substances and with hypodermic administered glycogen.

L.M. Mirtova (Moscow) presented experimental material on the regeneration of peripheral nerve in monkeys and other animal species in ontogeny. In experiments on monkeys, rabbits, guinea pigs, white rats and roosters in early periods after birth and in the adult state, regeneration characteristics have been established

for the sciatic nerve depending on the species and age of the animal.

Yuan Li-Yun (Leningrad) spoke on the change of nucleic acids with physiological and repair regeneration of the tongue mucous membrane epithelium in white mice. Considerable variations of quantities and distributions of nucleic acids in nuclei and in cytoplasm of undamaged and regenerating epithelium elements were histochemically determined. The nucleus plays an active role in this process.

In the discussions (A.N. Studitskiy, I.D. Liozner and S.I. Shchelkunov) the work perspective was emphasized and the desires for further research in this direction were expressed.

In the Fifth Session M.F. Merkulov (Moscow) in the paper "Insertion of labelled amino acids into proteins as a rejuvenation rate indicator in structural elements of the tissues" emphasized that the restoration of cell and tissue structures is closely related with protein synthesis intensity. But the administration rate of labelled amino acids into the organism tissue proteins can be

can be divided into several groups. The largest amounts of labelled amino acids are introduced into cells which produce protein secretions and which are characterized by a high rate of cell regeneration. Cellular elements, which do not produce protein secretions, and in which cellular division rarely occurs, have a considerably low level of labelled amino acid pick-up. Into non-cellular structures the labelled amino acids are introduced at the lowest rate.

A.A. Manina and L.I. Chekulayeva (Leningrad) reported on the radioautographic study of temperature factor effect on protein metabolism and physiological regeneration. In experiments on 10 day old and adult rats sharp differences of metabolic reactions were exposed in the nervous system and in the skin. Variations of outside temperature were distinctly reflected on the self-regeneration rate of proteins in the epidermis: with temperature increase self-regeneration was reinforced, with decrease - was reduced.

P.P. Rumyantsev (Leningrad) devoted his report to morphophysiological groups of the ability of frog

myocardium to full valve regeneration. After complete transverse crushing of heart ventricle in grass frogs a real regeneration of myocardial fibers was observed through myosymplast stage. Functions of conducting sinus rhythm and contraction are gradually restored.

Report of I.A. Poberiy (Moscow) "Study of regeneration processes in blood-producing organs by microautoradiography method" concerned the determination of labelled methionine introduction rate into the structures of different blood elements. Three hours after the administration of labelled methionine its incorporation is noted principally in cells which are little differentiated. After 24 hours the difference in labelled amino acid contents in young and mature blood elements is unimportant. The mitotically dividing cells incorporate the labelled methionine less than the non-dividing ones.

S.T. Tsygankova (Moscow) reported on experimental results in paper "Regenerative processes in narrow after anematize of animals and administration of the blood replacing liquid". The introduction of hydrolysate TsOLIPK into anematized animals reinforces the regener-

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ation of red blood cells, and depresses the leukoblast growth. Improvement of general state of the animals is observed after 6-8 hours, instead of 2-3 days without hydrolysate administration.

A.M. Chernukh and P.N. Aleksandrov (Moscow) presented data of the relative effect of antibiotics on tissue regeneration in the inflammation site and on the development of the chick embryo. Penicillin and tetracycline stimulate the connective tissue cell proliferation processes in the inflammation site. They also increase the growth of chick embryos, but without stimulation of organ differentiation.

Lu Tseyan-Chan (Leningrad) appeared with the paper "Regenerative effects in uterus wall tissues of white rats with the introduction of foreign body". The author studied regenerative and inflammatory effects in the uterus of different aged rats, and on different stages of the sex cycle and pregnancy with stitching of the uterus wall with a thread. The inflammatory reaction is different in different age periods and with different functional conditions of the organ. Erythrocytic extra-

visation substances play a different role in phagocytic "resection".

O.T. Movchan, A.A. Braun, V.P. Mikhaylov, A.N. Studitskiy, L.N. Zhinkin, and S.Ya. Zalkind took part in the discussion of the reports.

The Sixth Session concerned regeneration problems related to radiation injuries.

V.P. Mikhaylov (Leningrad) in the review "Principle trends in the study of tissue regenerative processes after radiation damages" characterized the following three principle trends, according to which studies are conducted at the present time: 1) Repair of tissue injury caused by momentary but strong irradiation; 2) Post-traumatic tissue regeneration after radiation injuries; 3) physiological regeneration with the influence of small but long-acting doses of ionizing radiation. The study of regenerative processes after radiation injury is closely related with the solution of a number of general character radiobiologic problems.

In the paper by G.S. Strelin (Leningrad) "New information on the restoration of the hematopoietic

function in irradiated animals by transplanting marrow cells" bibliographic data were given, which had been discussed on the Second Geneva Conference on Peaceful Use of Atomic Energy. At present is determined that after major doses of irradiation acclimatization occurs even for heterologous cells. The course of radiation sickness in addition to this is improved. With time its own blood-producing function is restored.

Ye.V. Dmitreva (Leningrad) had spoken on experiments on post-traumatic regeneration of skeletal muscles under different X-irradiation conditions. It was shown by her that skeletal muscle fibers in rats with total-body direct and indirect X-irradiation (1,000 r) and local irradiation (6,000 r) maintain high regenerative capacity. The delay in regeneration with total irradiation is related to slow reabsorption of necrotic masses, and with local irradiation - to the injury of muscle fibers themselves and weak development of granulation tissue.

V.Ya. Karmysheva (Ryazan) presented experimental data concerning the reactive and regenerative processes in ovaries after the influence of ionizing radiations.

Reactive, destructive and restoring processes have a different course depending on the irradiation dosage and the radiation sickness periods. In addition to this the ovarian tissues possess different sensitivities to irradiation. The ovary, according to opinion of Karmysheva, is characterized by a high regenerative capacity after irradiation.

T.L. Sopova and N.Y. Smirnov (Leningrad) showed results of the research "Bone regeneration after its traumatic damage in acute radiation sickness". They studied the regenerative process in dog femoral bone. The regeneration in irradiated and control dogs ended at the same time. Together with that, a lesion aggravates the course of irradiation sickness. Regeneration occurs at the expense of endo and periostium cells, and also at the expense of mesenchyme cells of marrow and pericytes.

In the paper "Study of destruction and regeneration processes in hematopoietic system after radiation influence" N.F. Barakina (Moscow) presented information on the nature of blood injury with radiation sickness, and factors causing their restoration. With total and local

irradiation the same result in blood changes is observed. Restoration however, occurs considerably faster if only part of the body or limbs are irradiated.

I.M. Shapiro (Moscow) reported experimental results on the theme "Variations of mitotic cell activity and chromosome injuries as factors which limit tissue regeneration after radiation influence". It was shown in the corneal epithelium and in liver that interkinetic nuclei maintain a reserve of chromosomal damages. They appear during mitosis. Cells with radiation injuries die in I-III generations.

When the papers were discussed (V.V. Kozlov, L.N. Zhinkin, G.S. Strelin, A.A. Braun, B.F. Sidorova, N.S. Artem'eva, I.A. Utkin and S.Ya. Zalkind), the importance of regeneration study on the grounds of radiation injuries and the necessity to work further in this direction were emphasized.

In the conclusion of its work, the conference accepted a developed resolution. In it, specifically, the timeliness of the gathering of the conference on physiological regeneration was noted. This problem, having a

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direct relationship to the most important theoretical and practical problems of medical science, is being successfully developed in the Soviet Union.

A number of practical measures were planned by the conference participants with the purposes of further reinforcement of studies on the problem.

The need of regularly calling meetings on the problems of regeneration and cellular multiplication was noted in the resolution.

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